

Lipase, Enzyme Activity

Catalog	Unit
TBP0077-100KU	100 KU
TBP0077-500KU	500 KU

Product Details

Form: Freeze-dried

Solubility: Soluble in water and dilute buffer

Stability: -20° C; -4° F

Activity: 20,000-50,000

Protein: 90%

Unit Definition

The amount of enzyme causing the liberation of one micromole of glycerol per minute at 37°C and pH 7.0.

Assay Method

The assay is based on a 4-step enzyme-coupled reaction. In the final step of the sequence, a dye is formed which can be measured spectrophotometrically at 490 nm. The increase in absorbance at 490 nm is directly proportional to lipase activity.

Applications

Lipase (EC 3.1.1.3) hydrolyses emulsified triglycerides of the long chain fatty acids. Lipase is active at the interface between the oil drops and the aqueous phase. In normal serum the concentration of lipase is low. In acute pancreatitis and in pancreatic carcinoma a rise in serum lipase activity occurs, with a mean increase being 50 times that of normal values. A rise in the serum lipase content is also found in acute and chronic renal diseases.

Reagents

- 0.1 M Potassium phosphate buffer, pH 7.0.
- 0.05 M 4-Aminoantipyrine-HCl (12 mg/ml) in distilled H₂O.
- 0.05 M 1,7-Dihydroxynaphthalene (8 mg/ml absolute ethanol).
- 0.05 M Magnesium sulfate (6.02 g/1000 ml) in distilled H₂O.
- 0.05 M ATP (30.3 mg/ml) in distilled H₂O.
- Peroxidase solution (588 U/ml). Dilute in 0.05 M phosphate buffer, pH 7.0 to a final concentration of 588 U/ml.
- Glycerokinase solution (36 U/ml). Dilute in 0.05 M phosphate buffer, pH 7.0 to a final concentration of 36 U/ml.
- a-Glycerophosphate Oxidase solution (700 U/ml). Dilute in distilled H₂O to a final concentration of 700 U/ml.
- Triolein emulsion. Mix 300 mg triolein/10.6 g Triton X-100 and heat until a single phase appears. Add 90 ml of distilled H₂O and mix.
- Reaction mixture. Mix together 0.128 ml 4-Aminoantipyrine, 0.064 ml 1,7-Dihydroxynaphthalene, 0.128 ml peroxidase, 12.4 ml 0.1 M phosphate buffer, pH 7.0, 0.150 ml glycerokinase, 0.32 ml a-glycerophosphate oxidase, 0.375 ml triolein emulsion, 0.375 ml magnesium sulfate and 0.375 ml ATP. Equilibrate at 4°C for one hour before use. Stable for 8 hours at 4°C.

Calculation

$$\text{Activity (U/mg)} = \frac{(\Delta E_{490\text{nm}/\text{min}})(\text{Total Vol.})(\text{Enz. Diln.})}{(E_{490})(\text{Enz. Vol.})(\text{mg Enz./ml})}$$

$$E_{490} = \frac{(4.96 \text{ cm}^2)}{\mu \text{ mole}}$$

Note: (1 unit = 1000 pH units)

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